

Meristem Tissue Culture of *Dendrobium* Ng Eng Cheow

by

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Introduction

The conventional methods of vegetative propagation of monopodial orchids is by cuttings while sympodial orchids are propagated by the division of the pseudobulbs. Both the methods can be very slow if one desires to propagate a large number of plants from a single selected plant. In view of this, in recent years orchidologists have successfully propagated plants through the technique of 'meristem' culture. Through this method, one may start with a piece of meristematic tissue and on successful culture, obtain a large number of protocorm-like bodies which could be induced to differentiate into new plants or be made to proliferate further to obtain more protocorm-like-bodies.

The success in meristem culture in orchids will have a tremendous impact on the economics of orchid culture in Singapore. The production of large numbers of 'prize' plants will enable orchid enthusiasts to obtain them more easily and it will also enable the commercial nurseries to increase their plants and flower production, both for export and for local distribution.

Literature Review

The technique of meristem culture in orchids was initiated by Morel (1960) who successfully obtained healthy *Cymbidium* plants when he cultured apices of shoots of virus infected plants. Morel (1964) observed that in culture, the excised meristem first enlarged to form a protocorm-like-body which then developed into a plantlet or produced a small clump of several protocorms, each giving rise to a plantlet. He also observed that further proliferation of protocorms took place on sectioning and sub-culturing the protocorms.

Wimber (1963, 1965) and Kunisaki et al (1972) observed that the number of protocorm-like-bodies could be greatly increased if the meristems were cultured in liquid media and particularly if there was agitation of the liquid, making it possible to obtain many plantlets from a single isolated piece of meristematic tissue.

Scully (1967) mentions that preliminary attempts to culture *Cattleya* explants on solid media were unsuccessful and hence all cultures were agitated in liquid media. He also observed that explants from the same vegetative shoot produced protocorm-like-bodies after varying periods of time.

Kunisaki et al (1972) observed that absence of sucrose encouraged proliferation of protocorms while sucrose encouraged the differentiation of protocorms into plantlets.

The pH of the nutrient media is an important factor. Wimber (1965) using a media of pH 5.6 had good results while Jasper (1966) recommends a pH of between 4.8 and 5.5. Scully (1967) adjusted the pH of his medium to between 5.0 and 5.2.

Sagawa and Shoji (1967) and others have been successful with the meristem culture of *Dendrobium* though not much success has been reported locally.

The present paper briefly describes the success obtained in the case of *Dendrobium* Ng Eng Cheow, the parents of which are *D. Jacquelyn Thomas* and *D. Alice Spalding*. *D. Ng Eng Cheow* is a local hybrid done by Koh Keng Hoe Nursery. It first flowered on 5 May 1972 and the hybrid was registered in May 1973. This hybrid, having white flowers, was selected to work with as there is a shortage of white orchid flowers in Singapore.

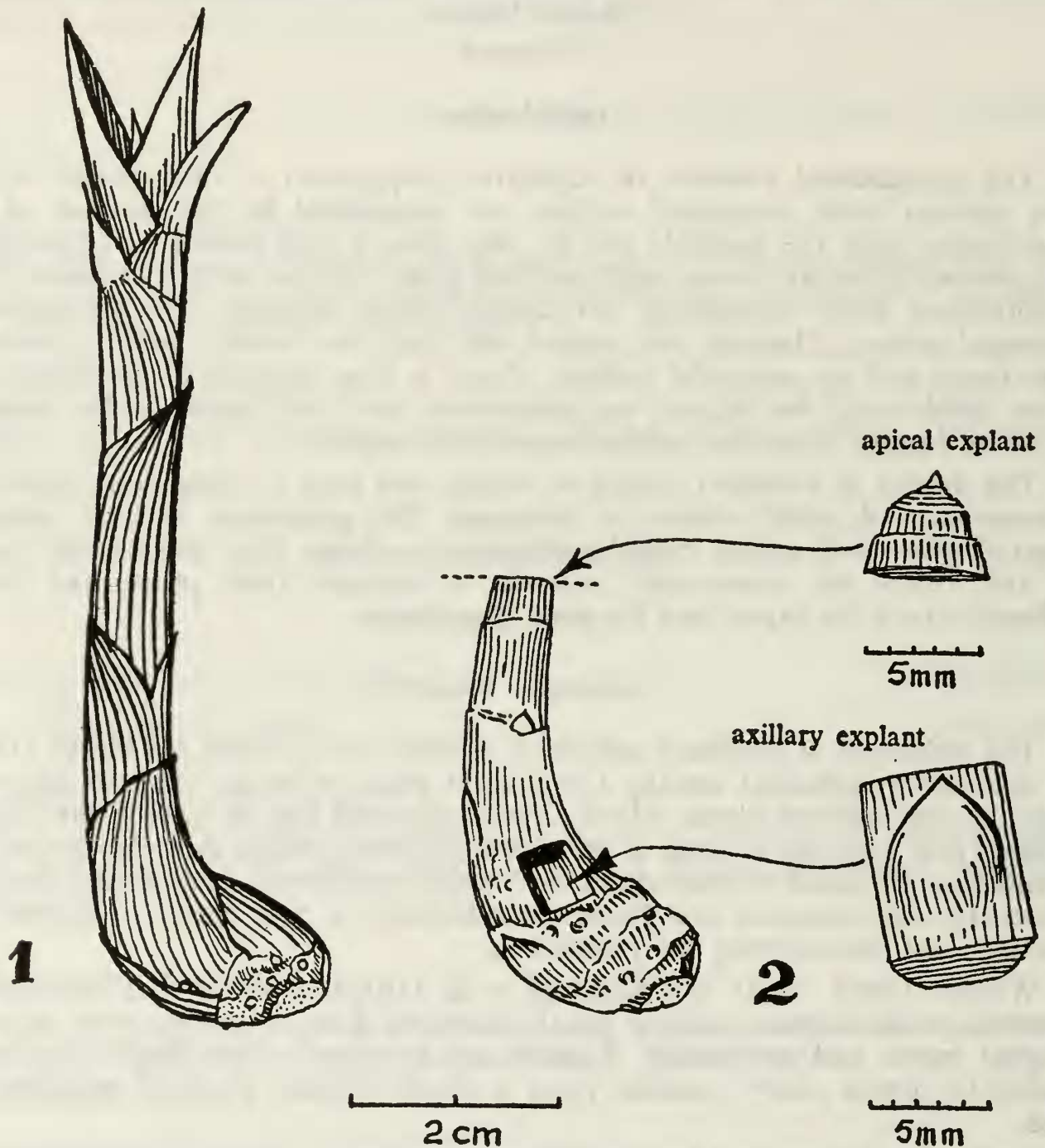


Fig. 1. A young shoot of *Dendrobium* Ng Eng Cheow.

Fig. 2. The apical and an axillary bud excised out from a shoot from which the enveloping leaves had been removed.

Design and Layout

Five culture media, namely Knudson C., Vacin and Went, Morel's Potato media, Murashige and Skoog and White's, which have been used by various workers in culture work, were selected to investigate their efficacies in relation to the propagation of *Dendrobium* Elizabeth. Both solid and liquid media were used and the treatments were arranged in a randomised block with five replicates for each treatment.

Meristematic buds found on young *Dendrobium* Elizabeth shoots were aseptically removed and the explant placed in liquid or on solid media. One explant was placed in each flask. Liquid treatments were mounted on an agitator to provide proper aeration of the plant tissue.

Explants were transferred to fresh media every two weeks and visual observations were made periodically (once every three days) to detect the emergence of protocorm-like-bodies.

Observation kept for a period of a month showed that explants in Vacin and Went's and Knudson C media showed a better response than those in the other three. It was decided to use Vacin and Went's medium as it appears to be the most popular in orchid tissue culture work. This medium was slightly modified in that ferric tartrate being found insoluble was substituted with ferrous sulphate.

Steward and Caplin (1952) observed that addition of coconut milk to the media caused a definite growth response in the cultures and thus 15 per cent of whole coconut milk was added to the media.

Morel (1965) observed the stimulating effect of Napthalene acetic acid on the growth rate of his cultures and thus this was incorporated in the media.

Young shoots (Fig. 1), about 8 cm long were removed from mature plants of *Dendrobium* Ng Eng Cheow. The leaves and leaf bases were removed from the shoot so as to expose the apical region and the axillary buds (Fig. 2), and this was then surface sterilized using a 10% solution of commercial Chlorox, for a period of fifteen minutes. Explants, about 5 mm cubes of tissue, which included the apical or axillary buds, were aseptically cultured in the modified Vacin and Went's medium (1949) with a pH of 5.0. The composition of the media is given in Appendix.

Explants were agitated at a rate of 40 times a minute and were exposed to continuous illumination of about 200 foot candles and a temperature of 21° — 25° C.

Results

Explants became green in 3–4 days' time showing an increase in size. In a month's time, explants had shown considerable growth and were transferred to media devoid of sucrose. Absence of sucrose encouraged proliferation of the tissue which started in about 8–10 weeks after transference.

Growth rates varied for the various explants from the same shoot even when subjected to the same experimental conditions. It was also observed that explants which included the axillary bud showed more growth potential than those which included the apical bud. After a period of 3 months, explants showed 3 distinct growth patterns.

1. Explants which developed into a single shoot (Fig. 3, Plate Ia).
2. Explants which developed into a shoot and also produced a few protocorm-like bodies at the basal portion of the shoot (Plate Ib). These protocorm-like-bodies differentiated into shoots, resulting in a cluster of shoots (Fig. 4, Plate IIa, IIb). Some of them showed the presence of roots. The cluster of shoots had to be separated to encourage further proliferation of protocorm-like-bodies from the basal portion of the shoot.
3. Explants which developed into a mass of protocorm-like bodies (Figs. 5, 6, Plate IIc, IId). These masses readily separated into a smaller groups and were very prolific in their growth. Some differentiation into shoots was seen. The clusters of protocorm-like-bodies were then separated into small groups (Fig. 7, Plate Ic) and cultured in solid media which contained sucrose but was devoid of napthalene acetic acid. The tissue showed

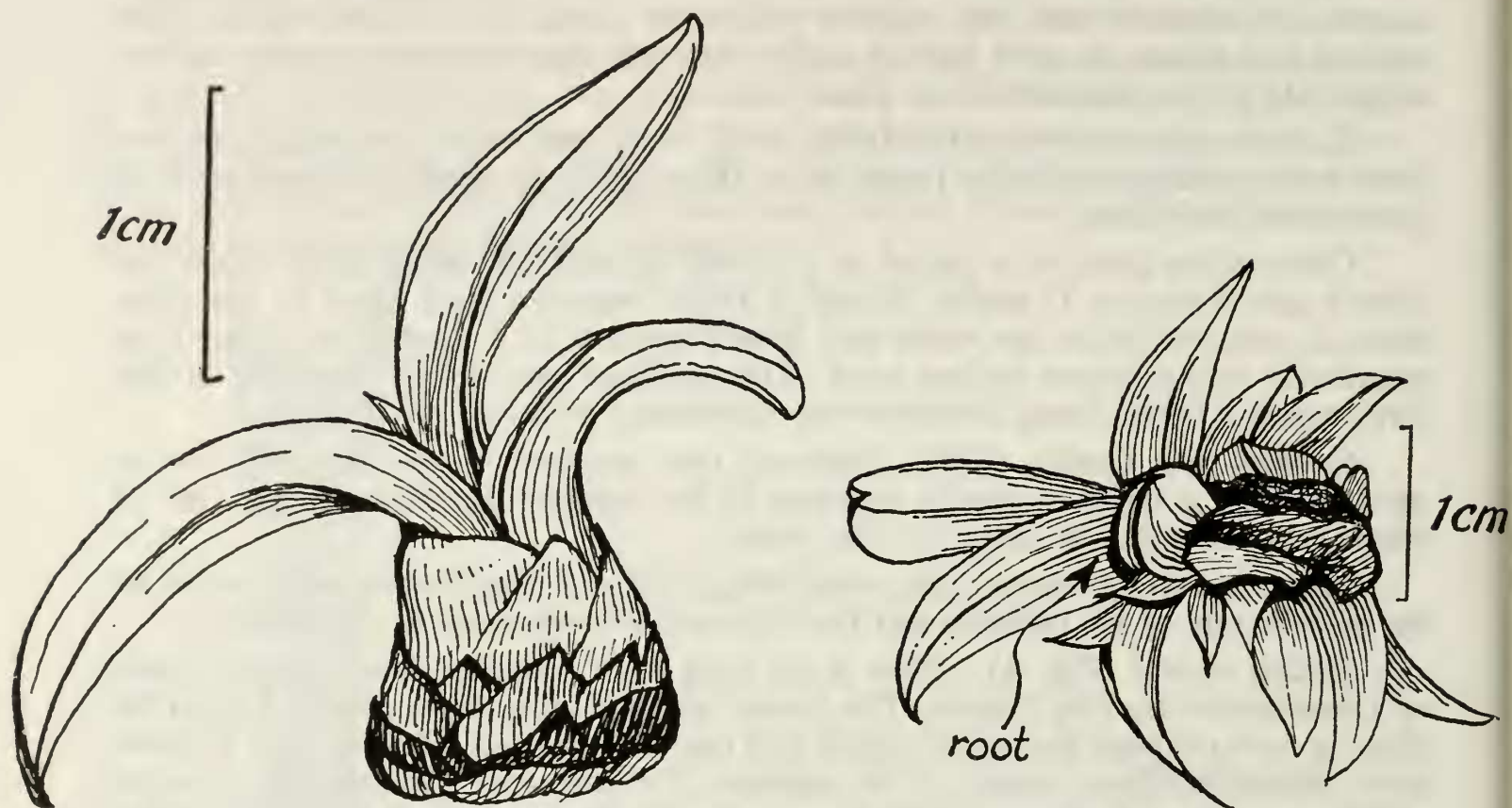
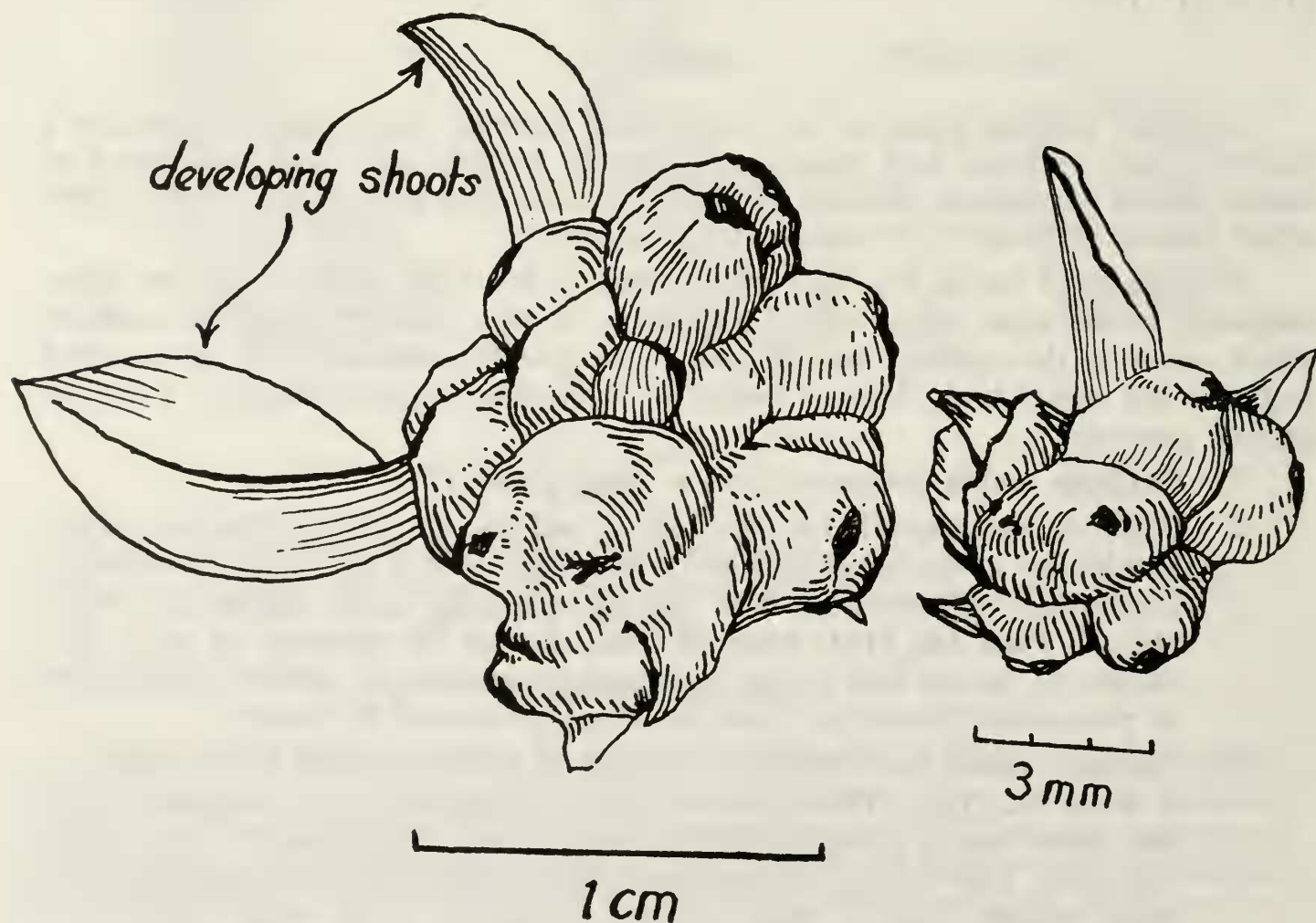


Fig. 3. (left): A single shoot which developed from the explant.

Fig. 4. (right): Multiple shoots which developed from the explant.



Figs. 5 (left) & 6 (right) A mass of protocorm-like-bodies which developed from the explant.

good response and distinct signs of differentiation were seen in 4 — 5 days' time along with an increase in size of the tissue mass (Fig. 8, Plate Id). The tissue mass increased considerably in size in 2 weeks' time and individual plantlets measuring about 5 mm in length were seen (Fig. 10, Plate IIIa, IIIb). At the end of 3 weeks, little protocorm-like-bodies were seen to develop at the bases of the existing plantlets, and these too started to differentiate into plantlets. Root primordia were also seen developing (Fig. 13). At the end of 4 weeks, distinct roots were seen and some plantlets had developed up to four leaves (Fig. 15, Plate IIIc).

Figures 7 to 15 (at the back) show the progressive differentiation of a mass of protocorm-like-bodies while Plate IV shows 10-week old plants growing in a community pot.

Protocorms were also cultured on the media (solid) given in the Appendix, which contained varying concentrations of sucrose and naphthalene acetic acid to determine which concentrations would induce maximum growth and differentiation of the protocorms.

Treatments	Sucrose	Napthalene Acetic Acid
A	—	—
B	1 gm/L	—
C	5 gm/L	—
D	10 gm/L	—
E	20 gm/L	—
F	10 gm/L	0.2 mg/L
G	10 gm/L	0.5 mg/L
H	10 gm/L	1.0 mg/L
I	10 gm/L	2.0 mg/L
J	10 gm/L	4.0 mg/L

The treatments were arranged in a randomized block with five replicates for each treatment.

Observations made two weeks later showed that in treatments *B* and *H*, individual plantlets having 2 leaves each had developed, while all other treatments had developed one leaf plantlets except treatment *J* in which no distinct leaves had developed, but instead more protocorm-like-bodies had developed from the existing ones. At the end of three weeks good plantlet development was seen in most of the cultures except in treatments *A*, *I* and *J*. The above observations showed that the presence of sucrose did encourage the growth and differentiation of plantlets and better growth was seen in media which contained lower concentrations of sucrose and naphthalene acetic acid when compared with media which contained the higher concentrations.

Conclusion

It was observed that explants, apical or axillary, behaved differently even when they came from the same parent shoot and were exposed to the same experimental conditions. This variation in growth perhaps was due to the age and size of the buds which were excised from the young shoots.

In a period of five months, the author has been able to produce several hundred plantlets from a single explant and it appears possible to produce several thousand plantlets in one year.

Acknowledgements

The author wishes to express his gratitude to Messrs Wong Yew Kwan and A. G. Alphonso, Commissioner and Deputy Commissioner of Parks and Recreation for their continued interest in the project and Messrs Pang Kim Whatt and Paul Loh for their technical assistance.

APPENDIX

<i>Media</i>		/L
Ammonium sulphate	0.50	gms
Magnesium sulphate	0.25	gms
Manganese sulphate	0.0075	gms
Potassium nitrate	0.525	gms
Mono potassium acid phosphate	0.25	gms
Tricalcium phosphate	0.20	gms
Naphthalene acetic acid	0.2	mg
Ferrous sulphate	0.028	gms
Sucrose	10.00	gms
Coconut water	150	ml

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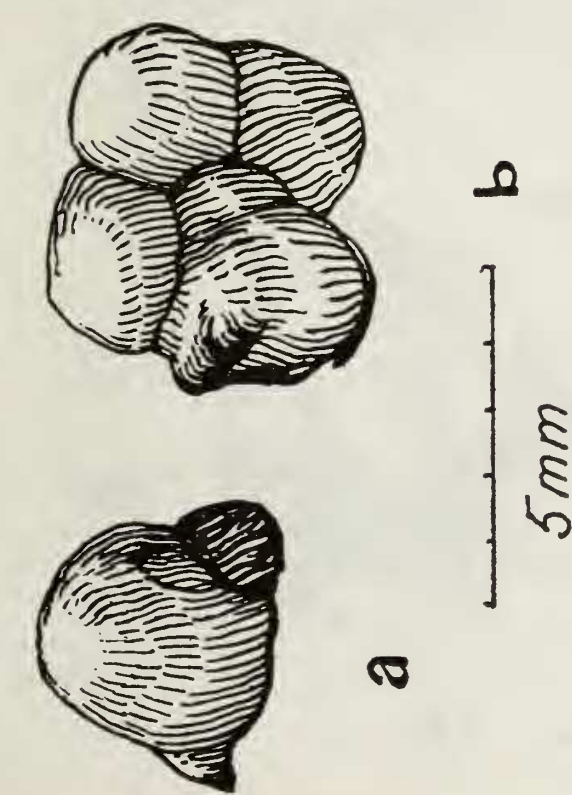


Fig. 7. 2 days after culture on solid media — proto-corm-like bodies.

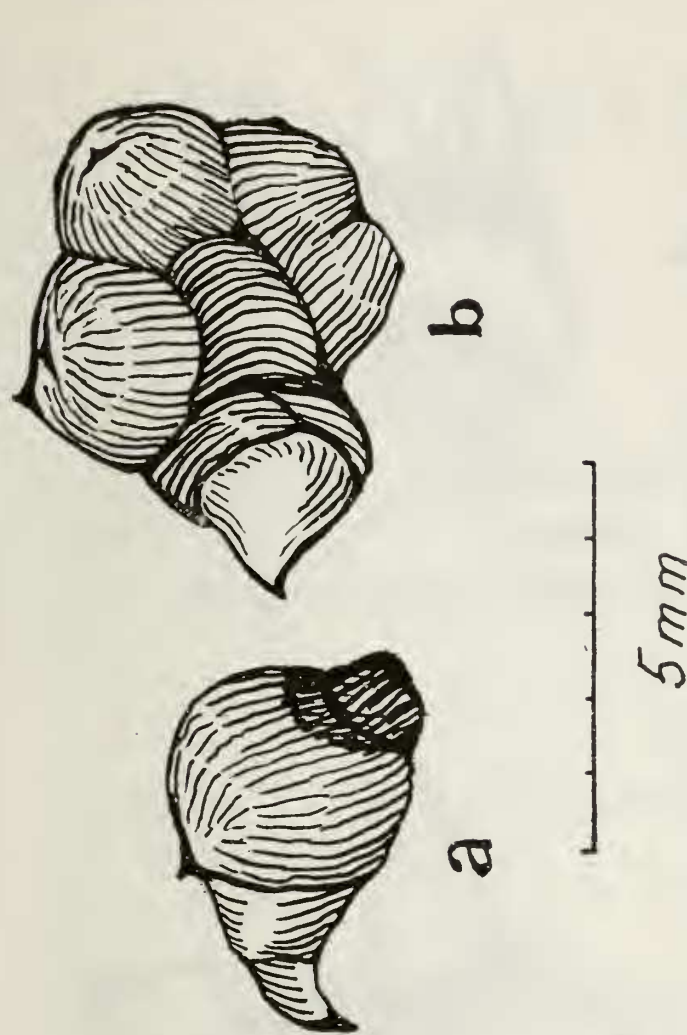


Fig. 8. 5 days after culture on solid media — tissue showing signs of differentiation.

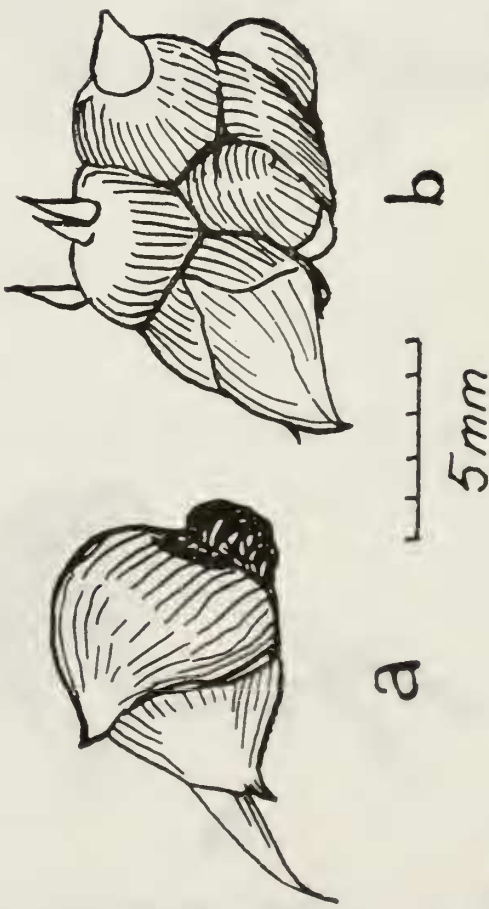


Fig. 9. 9 days after culture on solid media — differentiating plantlets.

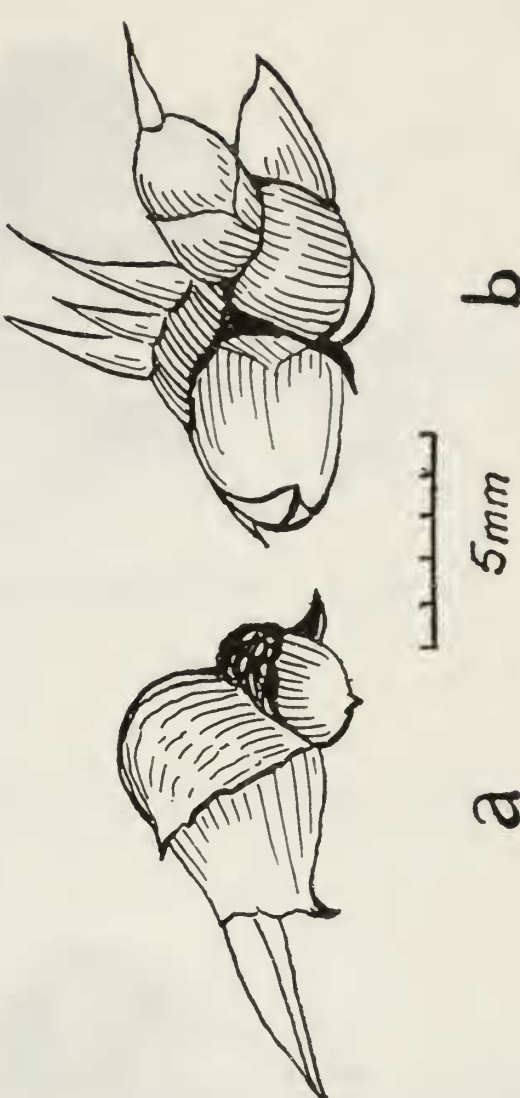


Fig. 10. 13 days after culture on solid media — plantlets measuring about 5 mm in length.

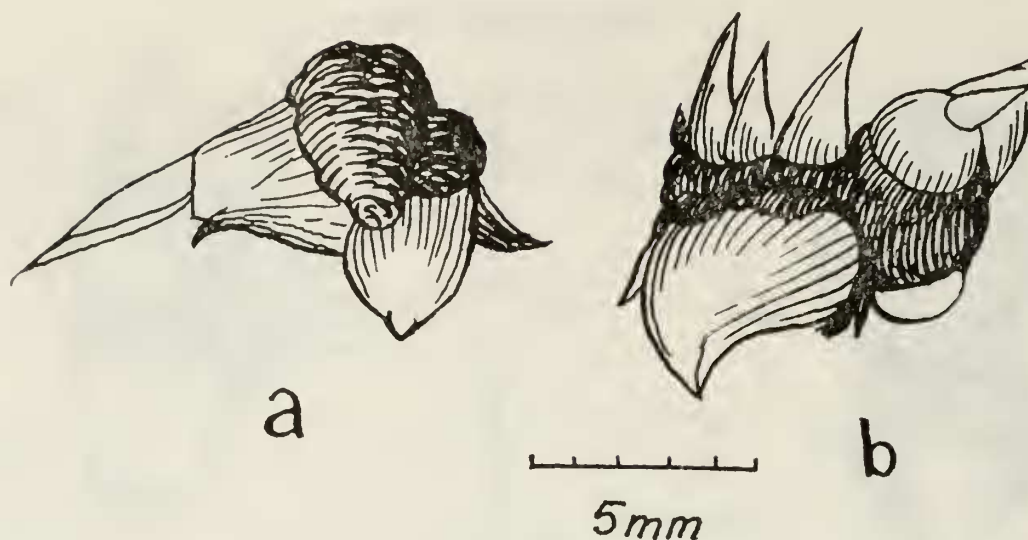


Fig. 11. 15 days after culture on solid media — developing plantlets.

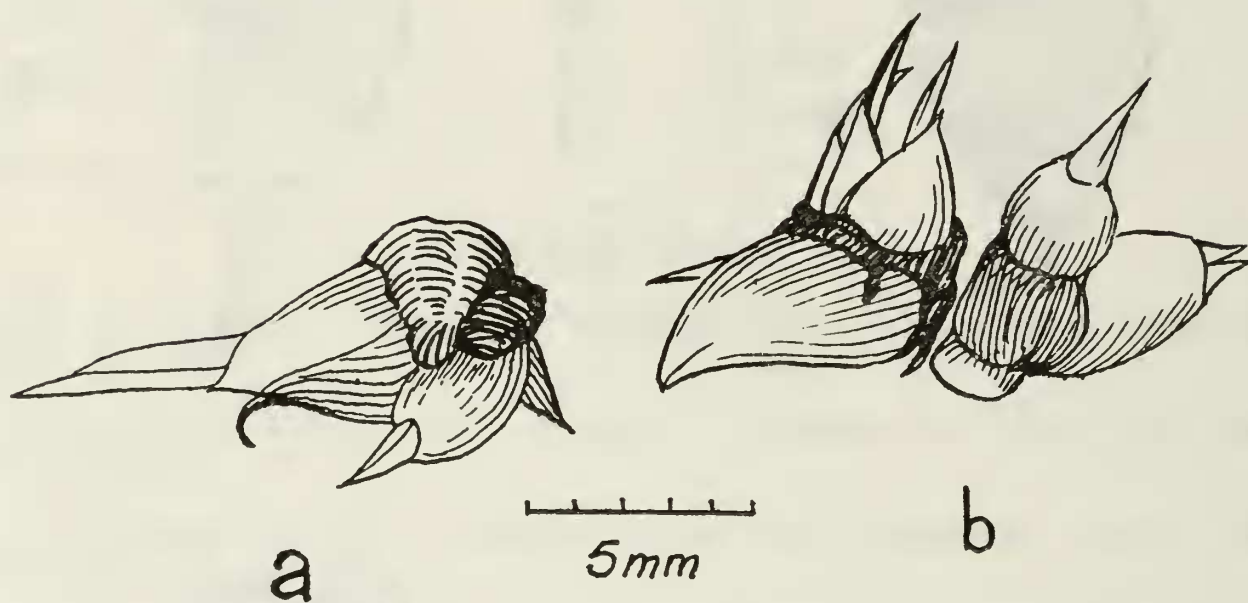


Fig. 12. 17 days after culture on solid media — developing plantlets.

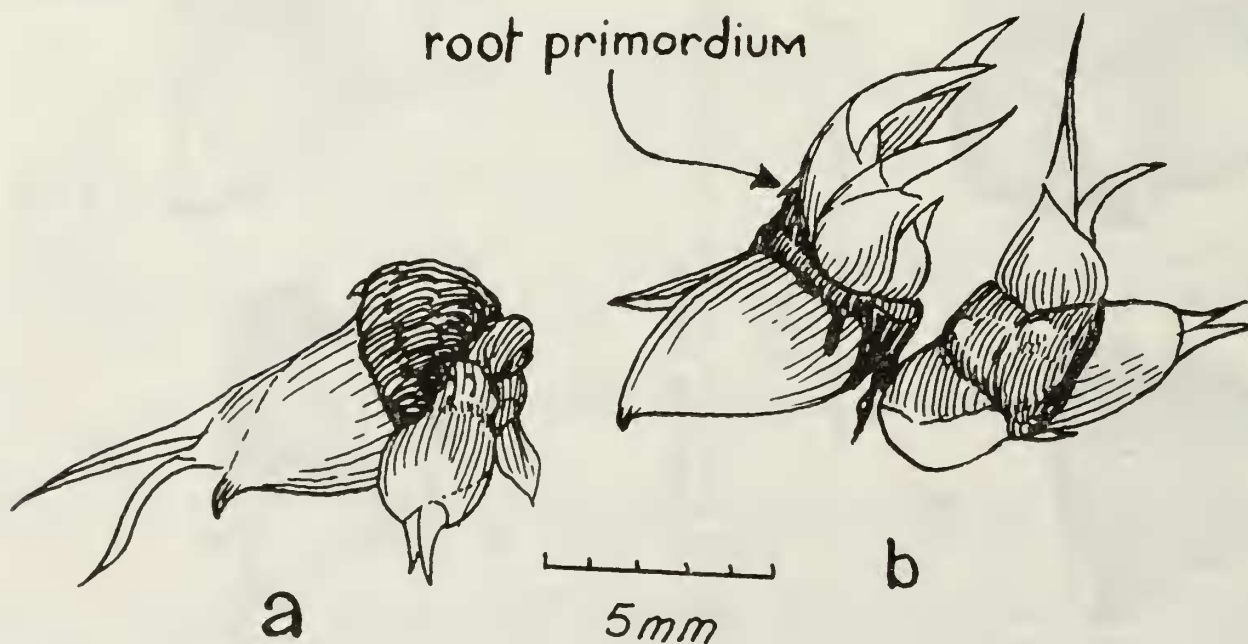


Fig. 13. 21 days after culture on solid media — root primordium developing from plantlet.

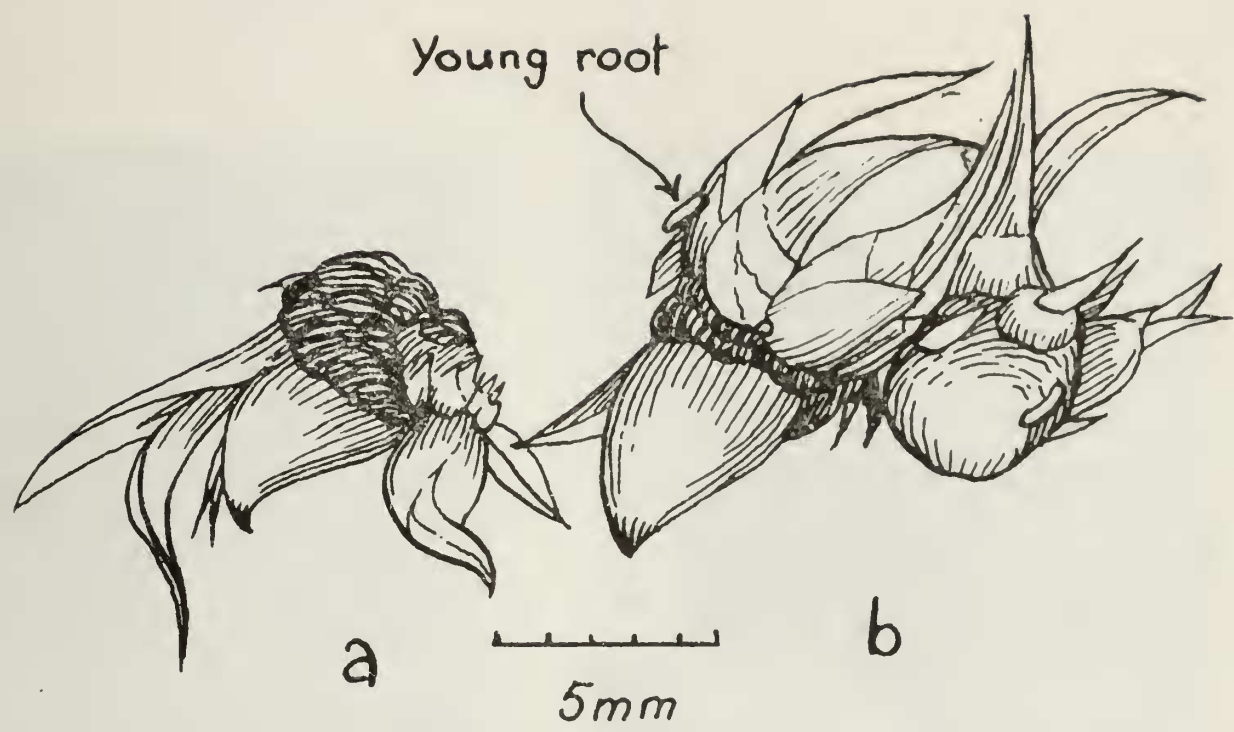


Fig. 14. 26 days after culture on solid media — plantlets showing developing roots.

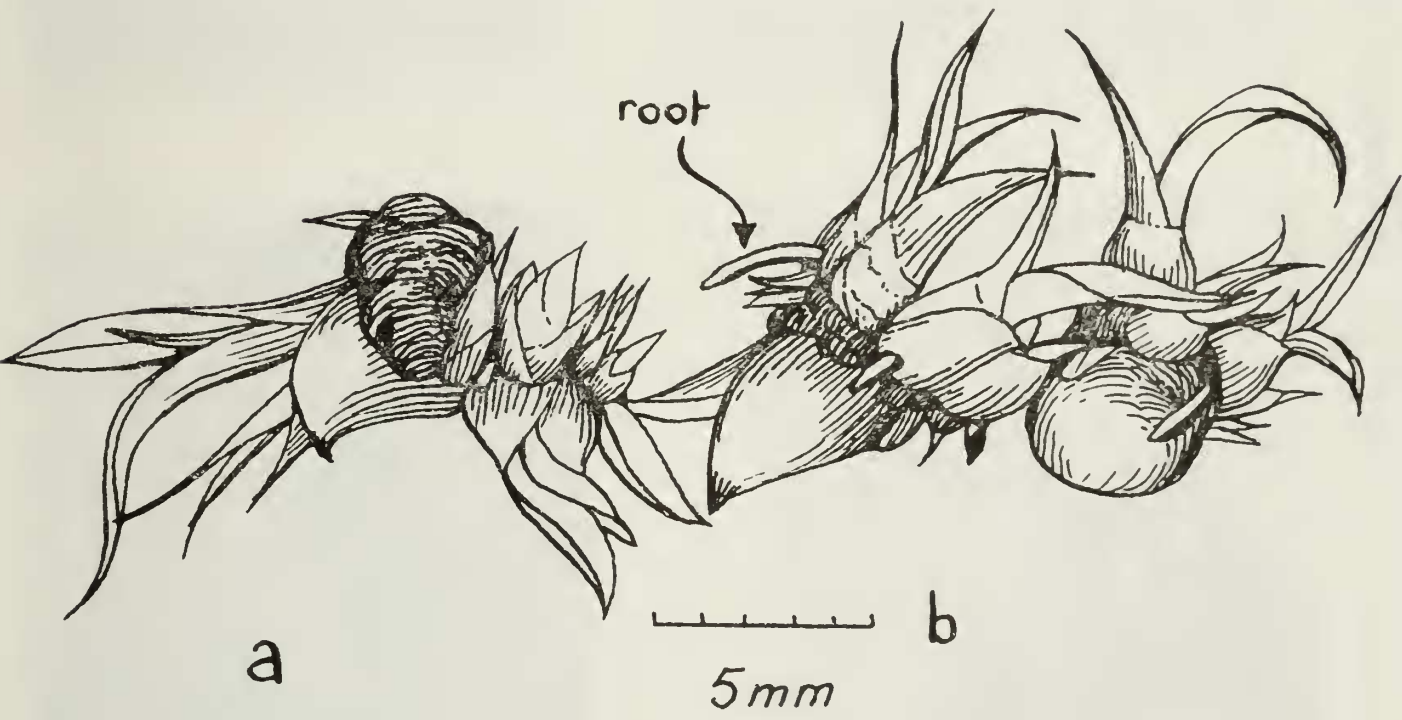


Fig. 15. 30 days after culture on solid media — young plantlets with distant roots.